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14. ABSTRACT: In this project, we propose to develop a new drug delivery vehicle based on dendrimer nanotechnology for personalized medicine. This new class of nanoplatforms contains imaging probe and molecular medicine with a cancer-specific targeting capability which is able to target cancer cells, monitor drug delivery and tumor response to achieve a "see and treat" strategy as a new concept of molecular medicine. Specifically, One Partner PI's lab will make dendrimers bearing functional handles to conjugate with chelating agents provided by the Initiating PI's lab for PET imaging and therapeutic peptides provided by another Partner PI's lab for the treatment of aggressive prostate cancer. To date, we have designed and synthesized the proposed bifunctional chelator scaffold system, CB-TE2A(^{t}Bu)$_2$-N$_3$ for the further construction of theranostic agents and multi-modality imaging probes for aggressive prostate cancer. In the meanwhile, we have designed a targeted theranostic small molecule drug conjugate (T-SMDC) system, and successfully synthesized a sample compound of T-SMDC, which consists of a PSMA-specific ligand, a PET imaging moiety, and a cytotoxic drug. The resulted T-SMDC retains the PSMA binding affinity and exhibits PSMA-dependent toxicity.

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INTRODUCTION

This project combines the recent advances in prostate cancer (PCa) research from three different labs integrated with a strong interest and dedication to develop a new molecular medicine approach towards the eventual cure of PCa. Like other cancer types, the current available therapeutic regimens for metastatic PCa are not PCa specific. With respect to PCa cells harboring various genetic alterations, the development of small molecular agents targeting these genetic defects to achieve a better therapeutic efficacy is foreseeable. In this project, we propose to develop a new drug delivery vehicle based on dendrimer nanotechnology for personalized medicine. This new class of nanoplatforms contains imaging probe and molecular medicine with a cancer-specific targeting capability which is able to target cancer cells, monitor drug delivery and tumor response to achieve a "see and treat" strategy as a new concept of molecular medicine. This platform system will be flexible to adopt any new cell targeting molecule or any therapeutic agents. Specifically, Dr. Simanek's lab will make dendrimers bearing functional handles to conjugate with chelating agents provided by Dr. Sun's lab for PET imaging and therapeutic peptides provided by Dr. Hsieh's lab for the treatment of aggressive PCa.

However, the failure of the multivalent strategy to deliver a biological effect that matches or exceeds the bioactive monomers have led us to prepare new monomers that might be more effective than those previously reported. The therapeutic sequence is maintained, but the targeting domain, and $(Arg)_{11}$ sequence is being replaced by the PSMA ligand called DUPA. During this period, the DUPA ligand (**Figure** 1) has been prepared and conjugated to a triazine linking group as shown below. The bioactive monomer has been prepared by solid phase synthesis and a chelating group has also be readied for creation of the final construct. Please Dr. Simanek's report for further details.

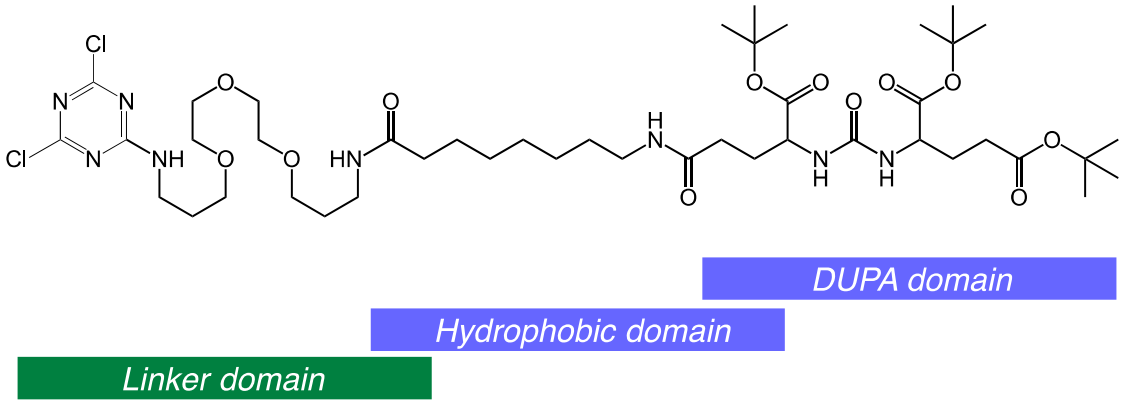


Figure 1. The DUPA domain subdivided to show the required hydrophobic domain and hydrophilic linker domain upon which the bioactive peptide can be appended.

BODY

Due to the targeting molecule change as noted above, we revised our Statement of Work as follows (Note: the corresponding task changes, the proposed work in the 2nd NCE period, are indicated with * asterisk.)

Aim 1: To construct dendrimer conjugates containing specific cell permeation peptides, peptide therapeutic(s) and a bifunctional chelator for PET imaging

Task 1 (Months 1 – 24): Synthesis and Characterization of Dendrimers - Scaffold Library (Simanek)

We will prepare dendrimers that 1a) Vary in size from generation 3-7 with an functional alkyne core (*Months 1 – 6);* 2b) Install a chelate for imaging at the alkyne site (*Months 4 – 12);* 1c) Survey surface groups for optimal behavior (*Months 9 – 18);* and 1d) Install therapeutic peptides for assessment (*Months 12 – 24).*

*No-cost extension (Months 48 – 60): Construct a prostate-specific membrane antigen (PSMA) targeted conjugate using a PSMA ligand, 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid (DUPA: *Ki* = 8 nM) for PCa theranostics.

Task 2 (Months 1 – 12). Synthesis and Characterization of CB-TE2A-based Bifunctional Chelator (Sun)

An azide-modified form of CB-TE2A, CB-TE2A(^{t}Bu)$_2$-N$_3$, will be synthesized to conjugate with the alkyne-introduced dendrimers *via* the well-established "click chemistry" procedure.

Aim 2: To select potent compounds with screening systems based on specific mechanism(s) of action

Task 3 (Months 1 – 24). Selection of therapeutic peptides using high throughput assays (Hsieh)

A panel of 6 different prostate cell lines without DAB2IP expression will be used. For detecting EMT, we are will establish a biomarker expression assay and a migration assay. The potency of each dendrimer conjugate will be determined based on the same molar ratio of therapeutic peptide with the positive control.

Aim 3: To determine the biodistribution, pharmacokinetics, and potential cytotoxicity

Task 4 (Months 13 – 24): Radiochemistry and in vitro assay of the synthesized theranostic agents (Sun/Hsieh)

We will perform 4a) Radiolabeling of peptide-dendrimer conjugates with ^{64}Cu. Radiochemical purity will be assessed by radio-TLC and radio-HPLC (*Months 13 – 18; Sun);* 4b) *In vitro* stability assessment in fresh mammalian serum and assessed by radio-HPLC (*Months 16 – 24; Sun);* and 4c) Combination optimization of small peptides on PCa: We will first determine the IC_{50} based on high throughput assays and then assess the anticipated synergism using combination index (CI) analysis (*Months 12 – 24; Hsieh).*

*No-cost extension (Months 48 – 60): Perform Task 4 on the DUPA conjugates for PCa theranostics.

Task 5 (Months 9 – 30): In vivo and PET/CT imaging evaluation of the synthesized theranostic agents (Sun)

The in vivo evaluation will be carried out in SCID bearing subcutaneous PCa (PC-3 or LAPC-4) on the upper shoulder of each flank of animal; the imaging studies will be performed on a Siemens Inveon PET-CT Multimodality System when the tumor xenografts reach the proper size (about 100 mm^3). The animal models will be provided by Hsieh's lab.

*No-cost extension (Months 48 – 60): Perform Task 5 on the DUPA conjugates for PCa theranostics.

Aim 4: To evaluate the therapeutic efficacy using various pre-clinical models (Hsieh/Sun/Simanek)

Task 6 (Months 24 – 36): Preparation of dendrimer conjugates for Aim 4 (Simanek). See Task 1.

Task 7 (Months 18 – 30): Target validation of dendrimer conjugates (Hsieh)

For target validation, DAB2IP$^{-/-}$ model with distinct EMT and hyperplasia in prostate epithelia will be used. Control group will use the same dendrimer particle without the targeting molecule. By monitoring a panel of serum markers and animal body weight, the possible long-term toxicity will be assessed in liver and kidney.
*No-cost extension (Months 48 – 60): Perform Task 7 on the DUPA conjugates for PCa theranostics.

Task 8 (Months 25 – 36): Therapeutic efficacy of dendrimer conjugates (Hsieh/Sun)
To determine the efficacy of dendrimer conjugate in preventing PCa metastasis or eradicating metastatic PCa, two orthotopic models with early onset of lymph node metastases (LAPC-4 (AR+) and PC-3 (AR-)) will be used to represent PCa subtypes with different degree of aggressiveness. Five groups of treatment (3 single agents, 1 combination and one control without R_{11} conjugation) will be carried out with power analysis. Histology, immunostaining and western analyses will be employed to determine the efficacy of metastasis prevention. FDG-PET/CT prognostic scans will be performed to evaluate for the therapeutic efficacy.
*No-cost extension (Months 48 – 60): Perform Task 8 on the DUPA conjugates for PCa theranostics.

Justification: This NCE is requested to accomplish the unfinished tasks proposed in Aims 3 & 4 of the project, which reflects the down-stream nature of my part of work. Given the progress in the synthetic work (Dr. Simanek) and the screening of potent compounds (Dr. Hsieh), we have decided to go with an alternative approach for prostate cancer targeting. To promote the delivery of the theranostic to prostate cells, a DUPA ligand will be explored in this NCE period. A critical intermediate (shown below) has already been prepared at 200 mg scale. The DUPA domain targets prostate-specific membrane antigen (PSMA). In this intermediate it is linked to a spacer domain and a triazine linker. The linker domain, a dichlorotriazine, derives from the chemistry used to generate the multivalent dendrimers. The virtue of this linker is that each chlorine atom can be reliably replaced in sequential fashion. The proposed efforts are organized around introducing an imaging chelate and subsequent in vivo evaluation followed by the introduction of the therapeutic peptide. Multivalent constructs can be derived by reacting this intermediate with a dendrimer and subsequent elaboration of the remaining chlorine atoms with theranostic groups in a statistical or controlled fashion. Of great benefit, the valuable drug and imaging construct are incorporated in the final steps.

Key Progresses Made in Partner PI's Labs in the 3rd Year

Dr. Hsieh's lab: We have designed different chemical modification of small peptide and characterized the in vitro biologic activities of the peptides using several metastatic prostate cancer cell lines. Our data have shown the better activity of chemical modified peptide than prototype peptide. However, we did not observe any enhancement of activity after dendrimer conjugation, suggesting that peptide conjugation to dendrimer might have altered its structure. Overall, we conclude small peptide therapeutics remains a potential specific targeting agent. Nevertheless, there are still some issues regarding to drug delivery such as type of nanoparticle, chemistry of conjugation needed to resoved in order to achieve an efficient delivery.

Dr. Simanek's lab: We have explored two new strategies for the derivatization of dendrimers with peptides. Covalent attachment of peptides to dendrimers using a maleimide/thiol strategy has been introduced, but the construct lacks biological activity. Efforts to realize releasable materials derived from a triazinylhydrazone have produced a proof-of-concept dendrimer that is being written up, but the conjugation of peptides is proving elusive. To this end, we have explored the use condensation of 1,3-diketones. Manuscripts of both efforts are expected during

in 2016. In the course of these studies, an intrinsic fluorescence of these dendrimers has been identified and explored. These results have been submitted for publication. A phenomological study of the solubility behavior of the dendrimers produced in the course of delivering on the synthetic aim have also been published. Efforts ongoing have pivoted away from multipmeric constructs of the previously identified active monomer. While the results of these efforts are being reported to the literature, research goals now look to replace the cationic targeting domain described in the original lead with the prostate cancer specific targeting domain called DUPA.

Work accomplished in the 1st NCE period toward the completion of Task 4 & Task 5

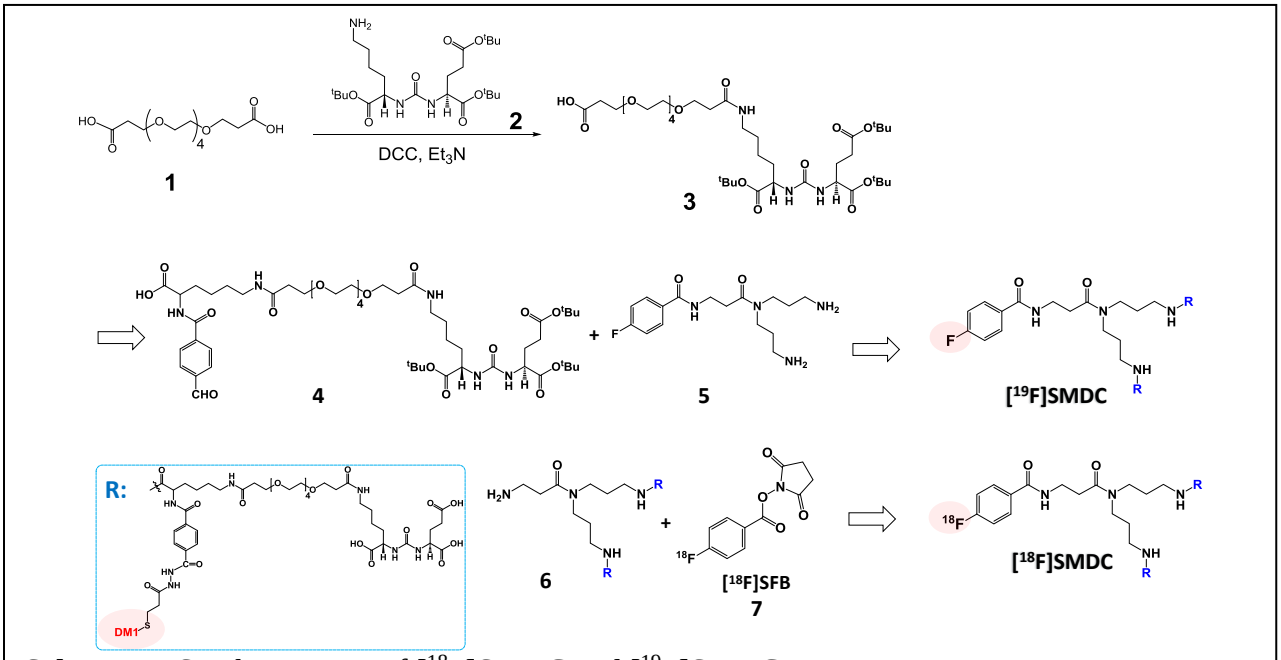
As stated in our last report, we have designed and successfully prepared a theranostic small molecule drug conjugate – T-SMDC which consists of tumor-specific ligand, PET imaging moiety, and cytotoxic drug. The resulted theranostic small-molecule drug conjugate (T-SMDC) remains the PSMA binding affinity and exhibits PSMA-dependent anti-cancer toxicity.

Given that the expected circulation half-life of our designed T-SMDC in vivo is likely within hours, the radioisotopes of choice can be either ^{18}F (97% β^+, $t_{1/2}$ = 110 min) or ^{68}Ga (90% β^+, $t_{1/2}$ = 68 min) for PET imaging.

RESULTS

Preparation of [^{18}F]SMDC and [^{19}F]SMDC:

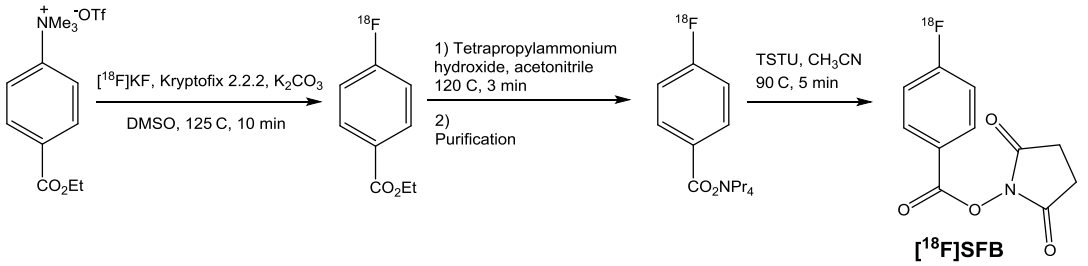
The synthetic route is shown in Scheme 1. The tetraethylene glycol based linker 1 was attached to the ε-amine handle of a PSMA ligand containing Lys-Urea-Glu 2, using carbodiimde chemistry to give a carboxy terminated PSMA targeting ligand 3 in 25% yield. Currently, we are working on the rest synthetic procedures to the designed [^{18}F]SMDC and [^{19}F]SMDC.



Scheme 1. Synthesis route of [^{18}F]SMDC and [^{19}F]SMDC.

Radiosynthesis of the [^{18}F]SFB:

The intermediate [^{18}F]SFB **7** will be needed to prepare [^{18}F]SMDC. We have established a reliable radiochemistry procedure to synthesize it on a GE Tracerlab FXN Pro module in a yield of ~44% (cartridge method) or ~18% (HPLC purification method) non-decay corrected. The radiochemical procedures are outlined in **Scheme 2**.

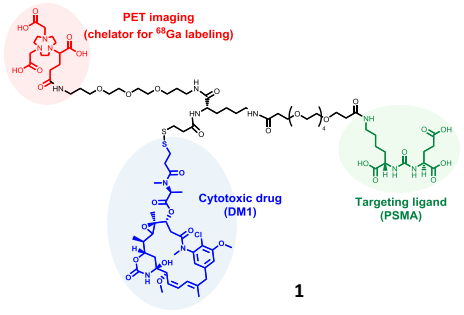


Scheme 2. Radiolabeling route of [^{18}F]SFB **7**

Preparation of [^{68}Ga]SMDC:

Recently, ^{68}Ga has gained considerable attention in the field of PET mainly because it is a generator (^{68}Ge/^{68}Ga) produced radioisotope. With an approximately one year of shelve-life, ^{68}Ge/^{68}Ga generators have been in the market for decades to make ^{68}Ga readily available as needed. Furthermore, gallium also has a long-lived isotope (^{67}Ga: γ, $t_{1/2}$ = 3.26 days), which has been in nuclear medicine practice for gamma scintigraphy and recently single photon emission tomography (SPECT). With identical chemical properties to ^{68}Ga, ^{67}Ga can be used instead of ^{68}Ga for *in vitro* assay or SPECT imaging as necessary. To date, a variety of bifunctional chelators have been developed for gallium radiopharmaceuticals including a NOTA chelator scaffold featuring its neutral complex formation with Ga(III) without compromising its stability and with a purposely added functionality for further conjugation. The chelator scaffold can form a stable complex as well with ^{64}Cu (17% β^+, $t_{1/2}$ = 12.7 hours), another common radioisotope to enable PET. This further indicates the versatility of our T-SMDC design.

An effective T-SMDC requires a highly potent cytotoxic drug to potentially overcome the dose difference between diagnostics and therapeutics, which is the main challenge facing the development of theranostics. For our work, the injected amount of T-SMDC must be controlled to be sufficient to ensure the PSMA-targeted therapy but under the level that may saturate the targeted PSMA binding sites. Because of the existence of PSMA-mediated internalization, such an injection can be administered multiple times if necessary, in particular to deliver a required dose for the targeted cancer cell kill. As such, one of the main determinants for a successful theranostics is the availability of a highly potent cytotoxic drug for the targeted disease. DM1, a cytotoxic maytansinoid that is too toxic to be used alone, is such a drug molecule for us to construct the first generation of our T-SMDC. Indeed, DM1 has been used to construct ADCs for cancer treatment. For instance, trastuzumab emtansine, an ADC with DM1 conjugated to trastuzumab, was approved in 2013 by US-FDA for clinical treatment of advanced HER2 positive breast cancer. In addition, DM1 possesses good aqueous stability and solubility, which is much needed for our T-SMDC design. For controlled release under reductive conductions within tumor microenvironment (e.g., glutathione, 1-10 mM), a disulfide linkage was

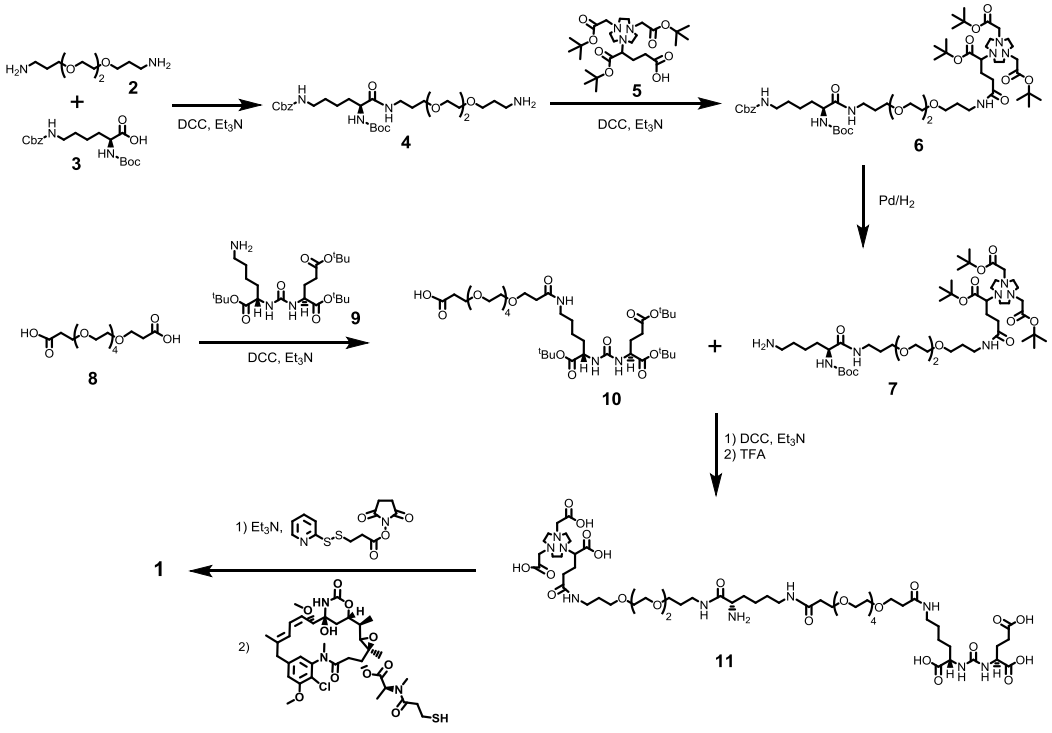


Scheme 3. Design of prostate specific membrane antigen (PSMA) targeted theranostic small-molecule drug conjugate.

introduced for DM1's anchorage at the α-amino position of the lysine center (**Scheme 3**).

To form a central frame, a PEG based di-amino linker **2** was conjugated using carbodimide coupling to the carboxylic group of a lysine derivative **3**, in which the two amino groups were orthogonally protected (**Scheme 4**). The product **4** was obtained in 22% yield after purification. It was then reacted with **5,** a NOTA chelator synthesized according to our published procedure, through its free side carboxy group to afford the fully protected **6** in 61% yield. The protecting group of ε-amine, carboxylbenzyl (Cbz), was removed from **6** via hydrogenation to give scaffold **7** in 91% yield for T-SMDC construction. Strategically the lysine center of **7** was chosen to provide two amino groups for the incorporation of the PSMA targeting moiety at ε-position and the DM1 molecule at the α-position. The presence of a free and one protected amine makes **7** a versatile and flexible scaffold for further T-SMDC construction.

In parallel, the tetraethylene glycol based linker **8** was attached to the ε-amine handle of a PSMA ligand containing Lys-Urea-Glu **9**, using carbodiimde chemistry to give a carboxy terminated PSMA targeting ligand **10** in 25% yield. The long linker separating the PSMA targeting ligand from the rest part of T-SMDC was chosen for its reported capability of retaining the PSMA binding affinity. The extended arm of ligand **10** carrying a carboxy group was coupled to the unprotected ε-amino of **7**. The resultant product was isolated and deprotected using trifluoroacetic acid to give **11** in 35% yield. Compound **11** had six carboxylic and one amino group at the α-position of the central lysine, which was intended for drug conjugation. In order to build in a disulfide linkage between the DM1 drug molecule and **11**, a commercially available succinimidyl 3-(2-pyridyldithio)propionate (SPDP) linker was first reacted with the α-amine in **11** to give an activated disulfide bond, which was readily converted to the desired T-SMDC **1** (NO3A-DM1-Lys-Urea-Glu) upon reaction with the thiol terminated DM1 drug molecule in 51% yield. Because of the instability of disulfide and high cost of DM1, the drug was incorporated to the entire T-SMDC at the last step.



Scheme 4. Synthesis route to T-SMDC **1**. Cbz: Benzyloxy carbamate; DCC: N,N'-Dicyclohexylcarbodiimide; TFA: Trifluoroacetic acid; Boc: tert-Butoxy carbamat

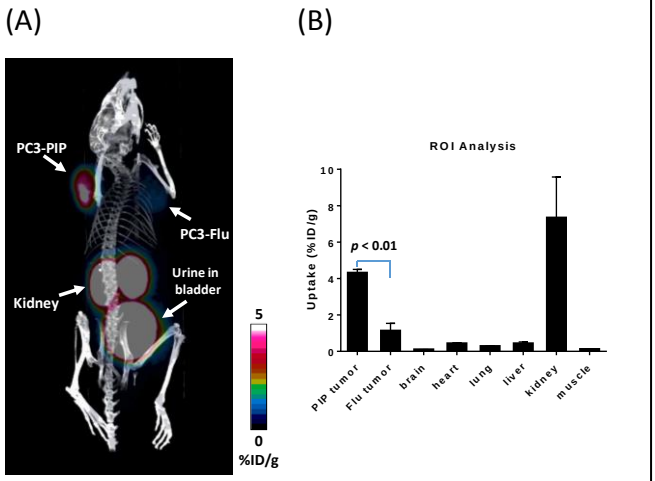
Radiolabeling and serum stability

NO3A-DM1-Lys-Urea-Glu (15 µg) was efficiently labeled by ^{68}Ga (148 – 222 MBq) within 15 min in quantitative yield as monitored by radio-HPLC when the reaction was carried out in 4 M NaOAc buffer (pH 4 – 4.5) at 60 °C. At lower temperatures, 25 or 37 °C, the radiolabeling was incomplete within 15 min. For both *in vitro* and *in vivo* evaluations, the radiochemical purity of ^{68}Ga-NO3A-DM1-Lys-Urea-Glu was maintained at > 95% as determined by radio-HPLC. The highest achievable specific activity was in the range of 50 – 80 GBq/µmol. Tested in human serum, ^{68}Ga-NO3A-DM1-Lys-Urea-Glu remained > 98% intact out to 3 h.

In vitro cell assays

To maintain the PSMA binding affinity of the Lys-Urea-Glu motif, a PEG-based spacer was introduced to separate the PSMA ligand from the rest part of T-SMDC. To verify the design, the PSMA binding affinity of T-SMDC **1** was measured by a competitive binding assay in LNCaP cells (PSMA+) using an ^{125}I-labeled Lys-Urea-Glu analog as the radioligand. Because the Lys-Urea-Glu analog has no available functional group for direct radioiodination, a Bolton-Hunter moiety was introduced. The specific activity of ^{125}I-**12** was over 74 GBq/µmol based on the HPLC measurement. The *in vitro* PSMA binding affinities of T-SMDC **1** were calculated by measuring the concentration of T-SMDC required to displace 50% of LNCaP cell bound ^{125}I-**12**. The free Lys-Urea-Glu ligand served as the positive control. T-SMDC **1** inhibits the binding of ^{125}I-**12** to LNCaP cells in a dose-dependent manner. A slight decrease of PSMA binding affinity was observed for T-SMDC **1** (IC_{50}: 187 ± 41 nM) as compared to the un-modified Lys-Urea-Glu ligand (IC_{50}: 96 ± 16 nM) indicating the PSMA targeting property of T-SMDC **1** was not significantly compromised by our chemical modifications.

The PSMA-mediated uptake and internalization of ^{68}Ga-NO3A-DM1-Lys-Urea-Glu were evaluated using LNCaP cells and PC3 cells. The non-specific uptake of ^{68}Ga-NO3A-DM1-Lys-Urea-Glu in LNCaP and PC3 cells was assessed in the presence of the Lys-Urea-Glu ligand at 1 mM. The total cell uptake versus non-specific uptake of ^{68}Ga-NO3A-DM1-Lys-Urea-Glu in LNCaP cells was significantly higher than in PC3 cells. When normalized to the cell numbers, the uptake in LNCaP cells becomes even higher than in PC3 cells. This observation indicates that the cell uptake of ^{68}Ga-NO3A-DM1-Lys-Urea-Glu is mediated by PSMA. As expected, ^{68}Ga-NO3A-DM1-Lys-Urea-Glu displayed an appreciable level of internalization in a time-dependent manner in the absence of Lys-Urea-Glu ligand. This serves as the mechanism for intracellular delivery of T-SMDC for PSMA-targeted imaging and therapy.



Scheme 5. (A) Representative PET/CT image of ^{68}Ga-NO3A-DM1-Lys-Urea-Glu in SCID mice bearing PC3-PIP (PSMA positive, left shoulder) and PC3-Flu (PSMA negative, right shoulder) xenografts (n = 3); (B) Quantitative organ uptake (%ID/g) derived from the PET images. ROI: regions of interest

Small animal PET/CT imaging studies were conducted in SCID mice bearing both PC3-PIP (PSMA positive, left shoulder) and PC3-Flu (PSMA negative, right shoulder) xenografts to evaluate the designed PSMA-specific imaging potential of ^{68}Ga-NO3A-DM1-Lys-Urea-Glu. The representative PET/CT images presented as the maximum intensity

projection (MIP) are shown in **Scheme 5**. As expected, the PSMA positive PC3-PIP tumor (4.30 ± 0.20 %ID/g) was clearly visualized at 1 hour post-injection (p.i.), while the contrast of the PSMA negative PC3-Flu tumor was barely above the background. The quantitative analysis of the images demonstrated a significant uptake difference of ^{68}Ga-NO3A-DM1-Lys-Urea-Glu in PC3-PIP (4.30 ± 0.20 %ID/g) and PC3-Flu (1.12 ± 0.42 %ID/g) tumors ($p < 0.01$; n = 3), indicating the anticipated PSMA imaging specificity of ^{68}Ga-NO3A-DM1-Lys-Urea-Glu. At 1 hour p.i., most radioactivity had been excreted into the urine from most of normal organs except for the kidneys. The observed high renal uptake (7.33 ± 2.25 %ID/g) resulted from the well-recognized PSMA expression in rodent proximal renal tubules, which is not of concern in humans. Liver, a major organ clearing the free DM1 drug, did not show a significant level of the radioactivity accumulation (0.42 ± 0.10 %ID/g), indicating a desirable in vivo distribution profile of the T-SMDC for reduction of the systemic toxicity of DM1.

In summary, we have designed a targeted theranostic small molecule drug conjugate (T-SMDC) system, and successfully synthesized a sample compound of T-SMDC, which consists of a PSMA-specific ligand, a PET imaging moiety, and a cytotoxic drug. The resulted T-SMDC retains the PSMA binding affinity and exhibits PSMA-dependent toxicity. When labeled with ^{68}Ga, the T-SMDC is capable of specific imaging PSMA-expressing cancer xenografts in mice. We recognize that the DM1 drug carried by the T-SMDC in the injected dose for PET imaging is low (*ca.* 0.1 nmol) to elicit the desired anti-cancer effects. In addition to taking advantage of the multi-valent NOTA scaffold for multi-presentation of DM1, we will consider three other approaches in our future work to realize the goal of the theranostic design concept for precision cancer patient care: i) increase the injection dose to the level that specific PSMA binding allows; ii) fractionate a therapeutic dose into multiple imaging doses. The PSMA-mediated internalization mechanism can be exploited for multiple administrations of an imaging dose until the desired DM1 dose is delivered, of which only one ^{68}Ga-NO3A-DM1-Lys-Urea-Glu dose is needed while others are its cold gallium counterpart to avoid unnecessary radiation exposure; iii) have PET imaging with ^{68}Ga-NO3A-DM1-Lys-Urea-Glu only serve the companion purpose for precision chemotherapy of DM1 delivered by the T-SMDC formulated with cold Ga(III). It is noteworthy that the T-SMDC design is versatile in that it can be applied to other targeting systems for developing theranostics of the corresponding diseases.

KEY RESEARCH ACCOMPLISHMENTS

- We have applied the proposed bifunctional chelator scaffold system to radiolabeling of antibodies with ^{64}Cu via a facile click-chemistry strategy (**published**).
- We have applied the chelator scaffold design to molecular design of targeted dual-modality imaging probes (**published**).
- A theranostic drug conjugate for prostate cancer has been successfully designed and synthesized with the integration of a PET imaging functionality (**published**).

REPORTABLE OUTCOMES

1. Lo S-T, Kumar A, and Sun X: Delivery and controlled release of therapeutics via dendrimer scaffolds. Chapter 10 of "*Nanoparticle Delivery of Biotherapeutics*" edited by Vooght-Johnson. Published by Future Science Group, **2015**

2. Kumar A, Zhang S, Hao G, Hassan G, Ramezani S, Lo S-T, Sagiyama K, Takahashi M, Sherry AD, Oz OK, Kovacs Z, and Sun X: Molecular Platform for Design and Synthesis

of Targeted Dual-modality Imaging Probes. *Bioconjugate Chemistry*, **2015**, 26(3):549-558. ***ACS Editor's Choice***

3. Kumar A, Hao G, Liu L, Ramezani S, Hsieh JT, Oz OK, and Sun X: Click-Chemistry Strategy for Labeling Antibodies with Copper-64 via a Cross-Bridged Tetraazamacrocyclic Chelator Scaffold. *Bioconjugate Chemistry*, **2015**, 26(4), 782-789.

4. Kumar A, Mastren T, Wang B, Hsieh JT, Hao G, and Sun X: Design of a Small-Molecule Drug Conjugate for Prostate Cancer Targeted Theranostics, Bioconjugate Chemistry, **2016**, 27:1681-1689.

CONCLUSION

We have designed and synthesized the proposed bifunctional chelator scaffold system, CB-TE2A(^{t}Bu)$_2$-N$_3$ for the further construction of theranostic agents and multi-modality imaging probes for aggressive prostate cancer. In the meanwhile, we have designed a targeted theranostic small molecule drug conjugate (T-SMDC) system, and successfully synthesized a sample compound of T-SMDC, which consists of a PSMA-specific ligand, a PET imaging moiety, and a cytotoxic drug. The resulted T-SMDC retains the PSMA binding affinity and exhibits PSMA-dependent toxicity.

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